THE PHENOMENON OF MUTUAL AVERSION BETWEEN MONO-SPORE MYCELIA OF THE SAME FUNGUS (*DIAPORTHE PERNICIOSA*, MARCHAL). WITH A DISCUSSION OF SEX-HETEROTHALLISM IN FUNGI

BY

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IMPERIAL BUREAU OF MYCOLOGY
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By DOROTHY M. CAYLEY

(*John Innes Horticultural Institution, Merton, Wimbledon*).

(With Plates XIX, XX and Three Charts.)

Preliminary Note.

During the investigation into the life history of *Diaporthe perniciosa* (Marchal), a fungus which gives rise to a rapid wilt and "die-back" of stone fruits in this country, a new phenomenon has been observed, namely that of mutual aversion of certain monospore cultures of this organism when grown together on artificial media. So far as is known to the writer, this phenomenon of aversion between colonies of the same fungus has not been recorded hitherto[1], and although the investigation is far from complete, the results, as far as they go, were thought to be of sufficient interest to allow of the publication of this preliminary note.

Diaporthe perniciosa was first isolated by Marchal(18) while working on rots of stored fruits in Belgium, and an organism, morphologically similar, has been found to be one of the causal organisms of wilt and "die-back" of stone fruit trees in this country. A specimen of "die-back" and a pure culture of *Diaporthe* from plum were submitted to Professor Marchal, and he is of the opinion that the two organisms are the same. Inoculation experiments have shown that *Diaporthe* from stone fruits can cause rot of apples very similar to the rot caused by a pure culture of *Diaporthe* obtained from Professor Marchal.

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The observations recorded in this communication were made on *D. perniciosa* isolated from diseased tissues of stone fruit trees.

D. perniciosa is an Ascomycete, belonging to the Valsaceae group of the Sphaeriales, and has two distinct stages, (1) the pycnidial or *Phomopsis* stage and (2) the perithecial or *Valsa* stage[1].

The pycnidia are formed under the bark of the host, and contain two kinds of spores, the oval viable "*a*" spores, as designated by Diedicke(9) and the filiform, non-viable, or "*b*" spores.

There is considerable divergence of opinion as to whether the "*b*" spores are typical spores, or only paraphyses or "basides" as held by Marchal. Repeated attempts to germinate these "*b*" spores have failed, and as far as can be seen they do not appear to have any definite nucleus. In *D. perniciosa* the "*b*" spores are formed first, later both "*a*" and "*b*" spores are present in the same pycnidium and finally "*a*" spores only.

The perithecia either develop in the same stroma as the pycnidia, or independently in the more deeply seated cortical tissues of the host. The perithecia contain numerous asci with eight bicellular ascospores.

The pycnidia and the necks of the perithecia burst through the bark, forming lenticel-like slits.

Considerable difficulty has been experienced in inducing *D. perniciosa* to pass through all the stages of its life cycle in pure culture on an artificial medium. Cultures from mass infections with pycnospores, and mono-pycnospore cultures develop the typical pycnidial stage with "*a*" and "*b*" spores very readily on a number of different media, but typical perithecia have only been found in three pycnospore cultures, one of which was a mono-spore culture, SS_1, and then only sparingly after many months' growth. Inoculations with this mono-spore culture have given rise to typical "die-back" of plum, and this strain has developed perithecia freely on the host plant.

Ascospore cultures, on the other hand, have given very variable results. Among mass platings of ascospores, some gave the typical pycnidial stage with "*a*" and "*b*" spores, others developed perithecia only. Mono-ascospore cultures develop perithecia in large numbers, but few or no pycnidia, and if pycnidia occur at all they contain rather undersized oval spores; no filiform spores have been found in mono-ascospore cultures.

It was concluded from these results that *D. perniciosa* might be

[1] A full description of the morphological characters of the fungus is given in a paper on "die-back" disease of stone fruits. *Annals of App. Biol.* Vol. x. No. 2, 1923.

heterothallic, two strains being necessary to enable the fungus to complete all the stages of its life history in pure culture.

A considerable number of mono-ascospore cultures were then isolated.

Technique.

The technique employed for the isolation of all the mono-spore cultures was that elaborated by Carl de la Rue (8) as follows: Sufficient 30 % gelatine is poured into a petri dish to form a thin film about 1 mm. deep and allowed to solidify. Then five or six drops of sterile distilled water are placed at intervals on the surface of the gelatine. The drops of water should roll on the surface if the gelatine is in the right condition of firmness. A platinum loop-full of a dilute suspension of spores is then placed in each drop, and the drops spread thoroughly over the surface of the gelatine with a sterile glass spreader. A dummy objective marker, as designed by C. de la Rue, with an aperture of a 1/6 objective, is then fixed to the triple nose-piece of the microscope, after having been previously dipped in alcohol and flamed. The gelatine plate can be examined under a low power, and when a field is found containing a single spore, the nose-piece is swung round and the marker gently pressed into the gelatine. The field is then re-examined to see that no other spores are present in the circle marked, before the circle is picked out under a lens with a small sterile platinum loop and transferred to a slope of culture medium. The dummy objective must be flamed and allowed to cool between each spore.

When the mono-spore cultures have grown, pieces of mycelium of two, or at the most three, separate cultures can be placed on a plate of solid culture medium and grown on together in an incubator.

In some combinations it was found that the resulting colonies grew across the plate and intermingled freely (Pl. XIX, figs. 1, 3, 4, 7); but others showed a definite aversion, leaving a well-defined line of demarcation between the colonies (Pls. XIX and XX). In the latter case the growth of the surface mycelium is arrested along the line of demarcation, and although at first the hyphae submerged in the medium intermingle, later they become disorganised and finally die out. A thick-walled stromatic protective growth of mycelium forms at the edge of each colony on either side along the line of demarcation, in some cases accompanied by discoloration of the medium (Pl. XX, fig. 9). After a time the agar dries out and cracks along the line.

If two pieces of mycelium from one and the same averting mono-spore strain are tested together, the colonies will intermingle and show

no aversion (Pl. XIX, figs. 1, 3). If two or three different mono-spore mycelia are grown in the same plate, they may either meet—if all the strains are alike—(Pl. XIX, figs. 1, 3, 4, 7); or, two will meet and a third show aversion to the other two (Pl. XIX, figs. 5, 6, 8, 9); or, in some cases, there will be a general aversion between all three colonies (Pl. XX, figs. 2–6).

Since two pieces of mycelium from the same strain will meet when grown together on the same plate, although that strain has shown aversion towards other strains, and since one colony on a plate can show aversion towards two other colonies which meet, all three of which have been isolated from the same host, like must meet like, and unlike show aversion to unlike. For the same reasons, the line of demarcation cannot be due to staling of the medium, but rather, as the general appearance of the plates suggests, the secretion of some toxin, possibly volatile, by the mycelia. This toxin diffuses more slowly through the substratum, so that the mycelium submerged in the medium is not affected so soon as that on the surface. The nature of the toxin is not known; it shows no reaction to litmus.

The phenomenon of aversion between any two antagonistic strains has occurred on all the media so far used, irrespective of the depth of the medium, the distance at which the inocula are placed, or the age of the cultures when tested. A number of mono-spore cultures have been grown in pure culture for more than two years and subcultured when necessary, on various media, without showing any signs of loss of toxicity.

D. perniciosa grows and sporulates well on prune-juice and plum-decoction-agar with or without the addition of crushed oats; on crushed-oats-agar alone, or with the addition of cane sugar or 1 % glycerine; also on sterilised plum twigs. After preliminary tests on all the above media had shown that aversion can occur between any two given strains irrespective of the medium, the bulk of the tests have been made on crushed-oats-agar, a medium which has the advantage of being quickly and easily made.

Unfortunately, on account of the small size of the fruiting bodies and other technical difficulties, this fungus does not lend itself to critical cytological investigation; but the perithecium arises from a very definite coiled multicellular archicarp, the cells of which are multinucleate (Pl. XX, figs. 7, 8). No male organ has been found and the archicarp becomes disorganised as the perithecium develops. The early stages of the perithecium resemble those described in *Polystigma rubrum* (2).

After the disorganisation of the archicarp has set in, some of the

smaller hyphae in the interior of the young perithecium show a bi-nucleate condition, but it was not possible to determine whether this bi-nucleate condition had any special significance or whether it was only due to rapid cell division in young actively growing mycelium; neither could it be seen if these hyphae were given off from the multi-nucleate cells of the archicarp. No association of nuclei or pores in the cell walls have been observed in the cells of the archicarp. The formation of ascogenous hyphae and the development of the ascus could not be followed.

As the cytological investigation of this fungus proved to be so difficult and unsatisfactory, the experiment was continued on different lines with the hope of being able to prove by other than cytological means, how and when this segregation or splitting up into physiological strains occurs, whether it is vegetative or promiscuous, or whether it occurs at some definite stage in the life-history of the organism.

Behaviour of mono-spore mycelia from various sources.

A total of 319 mono-pycnospore and mono-ascospore cultures have been under observation for some time, and as regards the occurrence of strains on the different hosts the results have shown that aversion may or may not occur, as follows, between:

1. Mono-ascospore } mycelia from different species of host.
   „ -pycnospore }
2. Mono-ascospore } mycelia from different varieties of host.
   „ -pycnospore }
3. Mono-ascospore { mycelia from different perithecia } on the same
   „ -pycnospore { „ „ „ pycnidia } variety of host, but different trees.
4. Mono-ascospore mycelia from different perithecia on the same host and the same stem.
5. In all perithecia so far tested, no aversion has been observed between mono-ascospore mycelia from the same perithecium, with the exception of one mono-pycnospore strain SS_1, which developed perithecia. This SS_1 strain will be discussed more fully later (pp. 358, 359, 363).

In the majority of cases, aversion occurs between mono-mycelia from different hosts whether of the same variety, different varieties, or different species, but mono-mycelia isolated from such widely different hosts as apricot and plum have been found to meet; on the other hand averting strains have been found in different perithecia on the same

host (Chart I, Groups VI, VII, VIII), thus showing that the type of strain is irrespective of the variety of host. It is quite possible that the occurrence of averting strains on the same host may be due to multiple infection from two or more different sources, and not to the splitting up into physiological strains in the host plant. More than two strains have not yet been found to occur on the same host, when the spores have been taken direct from diseased tissues, but the results from the investigation of SS_1 have clearly shown that a mono-pycnospore culture is capable of splitting up into a number of averting strains in the first ascospore generation.

But it must be pointed out that although mono-mycelia show aversion when grown on an artificial medium, it does not necessarily follow that this is the case when the parasite is growing on the host. The host may absorb the toxin as soon as it is formed, and the diseased condition may be one of poisoning. There is no mechanical blocking of the vessels by the fungus itself. The mycelium is present chiefly in the medullary rays and cortical tissue. The vessels are frequently blocked by gummy deposits, but not in sufficient numbers to account for the sudden wilting of the whole part affected.

The development of mono-spore mycelia on artificial media.

This development can be divided into three distinct classes.

Class 1. Mono-pycnospore cultures obtained from pycnidia containing "*a*" and "*b*" spores, which appear to be incapable of forming perithecia.

Class 2. Mono-ascospore cultures capable of forming a reduced pycnidium with "*a*" spores only, and numerous mature normal perithecia.

Class 3. One mono-pycnospore culture SS_1 (mentioned above) obtained from a pycnidium containing "*a*" and "*b*" spores, which produced pycnidia with "*a*" and "*b*" spores freely, as in Class 1, but also, after many months, eventually produced mature perithecia with typical 2-celled ascospores. Mono-mycelia obtained from these ascospores have in their turn produced the normal pycnidial stage with both kinds of pycnospores. These cultures have not been growing long enough to show whether they will eventually produce a second generation of perithecia. They may lose their capacity of forming perithecia and revert back to Class 1.

These results are set out in tabular form in Table I.

Discussion of Table I.

Class 1. This class calls for no further comment, except that as a general rule mono-pycnospore mycelia from the same pycnidium show no aversion *inter se*, although one definite case of aversion has occurred (Chart III, SS_4 and SS_7). The investigation of mono-pycnospores is not sufficiently complete to allow of any more definite statement. However a number of mono-pycnospore cultures have been under observation for a considerable time, and have always remained the same whether sub-cultured from spores or pieces of mycelium.

Class 2. In this class, which comprises mono-ascospore cultures SA_{1-101} from different perithecia (Chart I, Groups I–XIII), in no case has aversion been found between mono-ascospore mycelia from the same perithecium. Moreover, the F_1 ascospore generations of the mono-ascospore averting strains SA_2 and SA_{19} have been fairly extensively tested, and it was found that mono-ascospore strains of the F_1 generation show no mutual aversion *inter se*—whether from the same perithecium or from different perithecia—(Chart I, Groups IX–XIII) or to their respective parents; but the progeny of strain SA_2 shows aversion towards that of strain SA_{19} (Chart I, Groups IX, X, XI towards Groups XII, XIII), and the progeny of each strain shows aversion towards the parent of the antagonistic strain. Thus, there is no splitting up into averting strains in the F_1 of this class (Pl. XIX).

Class 3. In this class, which comprises mono-ascospore cultures $SA_{108-110}$ and SA_{115}–SA_{134} and mono-ascus cultures $SA_{(111)}$–$SA_{(115)}$ (Chart II, Groups XIV, XV) there is a complete reversal of what occurs in Class 2. Groups XIV, XV are the F_1 ascospore generation of the mono-pycnospore culture SS_1 already mentioned, which proved capable of forming perithecia in the mono-mycelial condition. Not only is there splitting up into averting strains in the same perithecium (Chart II, Group XV), but the F_1 mono-ascospore mycelia of this strain also shows aversion towards the parent culture (Chart II, SS_1 and Groups XIV, XV).

It will be seen in Chart II that the majority of these mycelia in Groups XIV and XV show aversion *inter se*, but that a few meet; also some of the F_1 mycelia meet one or other of the 10 stock mono-pycnospore strains with which they have been tested.

If the hypothesis is correct "that like meets like and unlike shows aversion to unlike" any two meeting strains must be the same.

TABLE I.

Development of mono-spore mycelia in pure culture.

Class	Type of fruiting body from which the mono-spore mycelia have been obtained	Fruiting bodies developed by the mono-spore mycelia		Behaviour of the F_1 *mono-ascospore* mycelia from the *same* fruiting body		Remarks
				With the parent	With each other	
1	Pycnidia on bark of host plant containing both "*a*" and "*b*" spores 121 *mono-pycnospore* cultures tested	Pycnidia with "*a*" and "*b*" spores	No perithecia	—	—	No F_1 ascospore generation
2	Perithecia on bark of host plant *or* perithecia developed in *mono-ascospore* cultures 106 *mono-ascospore* cultures from 13 perithecia	No pycnidia *or* reduced pycnidia with "*a*" spores only	Numerous perithecia	No aversion (See Chart I)	No aversion	The F_1 *mono-ascospore* cultures have either no pycnidia, or reduced pycnidia with "*a*" spores only, but produce numerous perithecia 56 *mono-ascospore* cultures tested in the F_1
3	SS_1 *mono-pycnospore* culture from a pycnidium on bark of host plant containing both "*a*" and "*b*" spores	Pycnidia with "*a*" and "*b*" spores	Perithecia	Aversion, but a few meet. (See Chart II)	Aversion, but a few meet. (See Chart II)	The F_1 *mono-ascospore* cultures produce normal pycnidia with "*a*" and "*b*" spores. These cultures have not been growing long enough to develop perithecia 90 *mono-ascospore* F_1 cultures tested, of which 64 were isolated from the *same* perithecium. (See Chart II, Groups XIV, XV)

Chart III shows the behaviour of the 10 mono-pycnospore test strains towards one another.

Until the F_2 of Classes 2 and 3 have been investigated no explanation can be offered to fit the above facts. There certainly appears to be some correlation between the capacity for complete development of the life cycle starting from a single pycnospore and the occurrence of aversion in the same perithecium in the F_1 ascospore generation.

It has not been possible to isolate the eight spores from an individual ascus, to see if segregation occurs in one and the same ascus, but it must be noted that cultures $SA_{(111),(112),(113),(114)}$ and $SA_{(115)}$ are mono-ascus cultures (i.e. whole asci) and they behave in the same way as mono-ascospore (i.e. single ascospore) cultures towards other strains. If all the spores of one ascus were not the same, it is conceivable that the degree of aversion would be reduced and the line of demarcation less pronounced. So far no marked difference has been observed, but as this point has not been critically investigated little stress can be laid upon it.

The above results definitely prove that certain mono-spore strains are capable of completing all stages in the life-history of the organism and others not. In the case of *Diaporthe perniciosa*, when dealing with the parasite on the host plant it has been noticed, in some instances, that when there is prolific pycnidial formation the development of a proportionate number of perithecia has not occurred.

These results also throw some light as to when and how the splitting up into physiological strains occurs in the life-history of this fungus, but the proof that certain strains can complete their life-history and others not, and that splitting up into averting strains can occur sometimes, but does not always, leads no further towards the elucidation of the fundamental question as to the constitutional differences of the various strains.

The strains in Class 2 differ from the one strain in Class 3 in that, in the latter class, the parent strain shows aversion to the majority of the mono-mycelia in its own F_1 ascospore generation, whereas in Class 2 the parent meets all mono-mycelia in its F_1.

This peculiar form of antagonism is quite unlike all known cases of sex-heterothallism in fungi in general, such as in the Mucors (3–7), Ustilaginales (15, 16, 17), Coprini (19, 20), and in *Aleurodiscus* and other Hymenomycetes worked out by Kniep (12–14, 16, 17). In *Diaporthe* it may only be a case of physiological strains and may have nothing to do with sex. The difficulty remains however, as suggested earlier, that antagonistic strains may be able to meet in the host plant. Experiments are

being devised to see if by infecting the same branch with two antagonistic strains an F_1 ascospore generation can be obtained different from that arising from infection with either strain alone.

Cases of sex-heterothallism have been recorded recently in the Ascomycetes. Dodge (10) found in *Ascobolus magnificus* that perithecia would not develop unless two mono-spore mycelia, properly chosen, were combined. This fungus develops male and female organs (archicarp and antheridium), but so far he has not been able to show whether any one mycelium consists entirely of one sex, producing archicarps only and another antheridia only. The mycelia intermix quite freely and numbers of sexual organs are found scattered throughout the hyphal mass, and not only in the region where the mycelia meet. Edgerton (11), working with mono-spore cultures of *Glomerella*, repeatedly isolated + and − strains, which when tested together, formed a dense line of perithecia where the strains met; but he also found that mono-spore mycelia when plated alone were capable of producing a few perithecia.

Explanation of Charts.

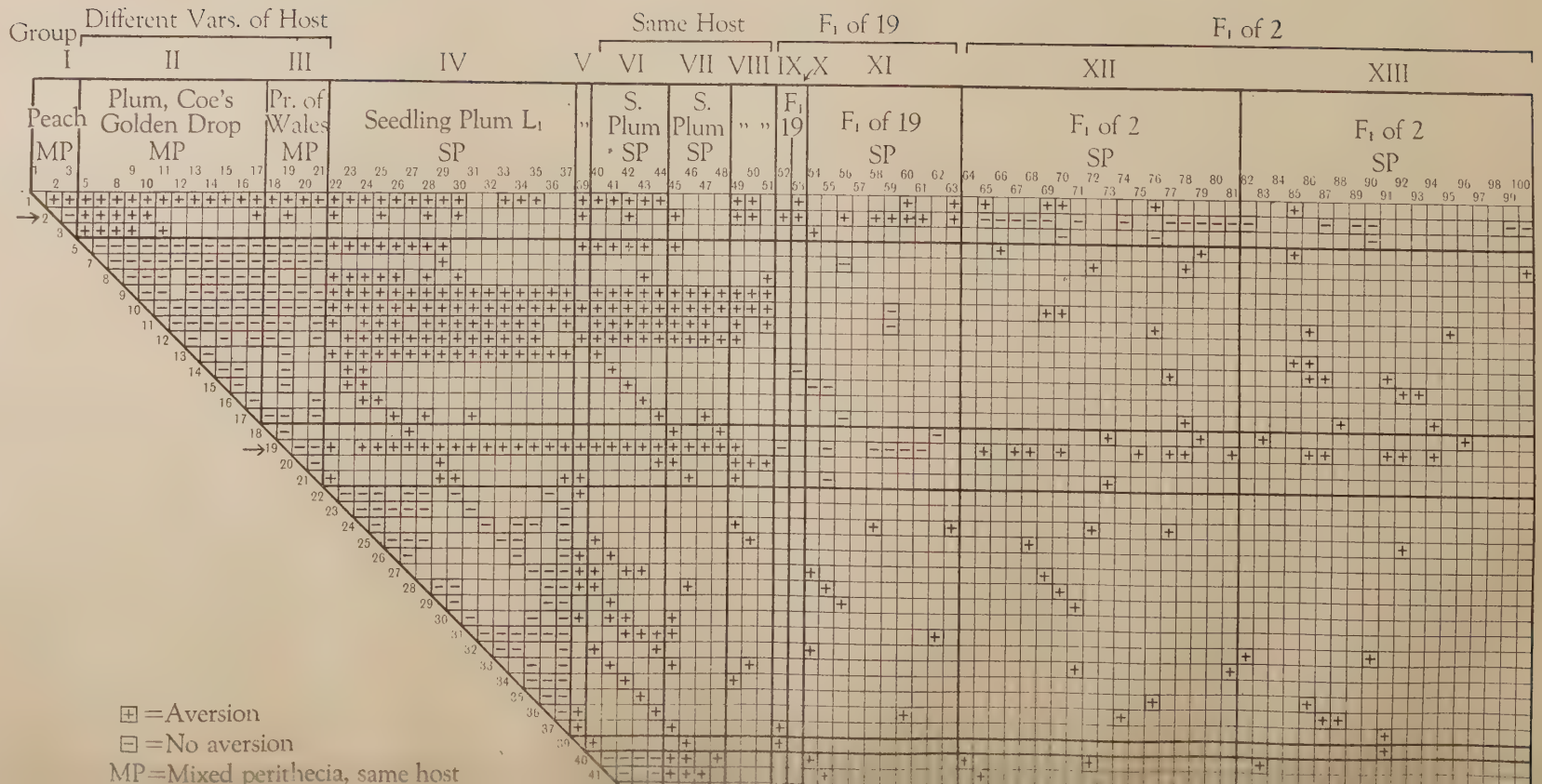
Chart I. (Class 2, Table I, p. 360.)

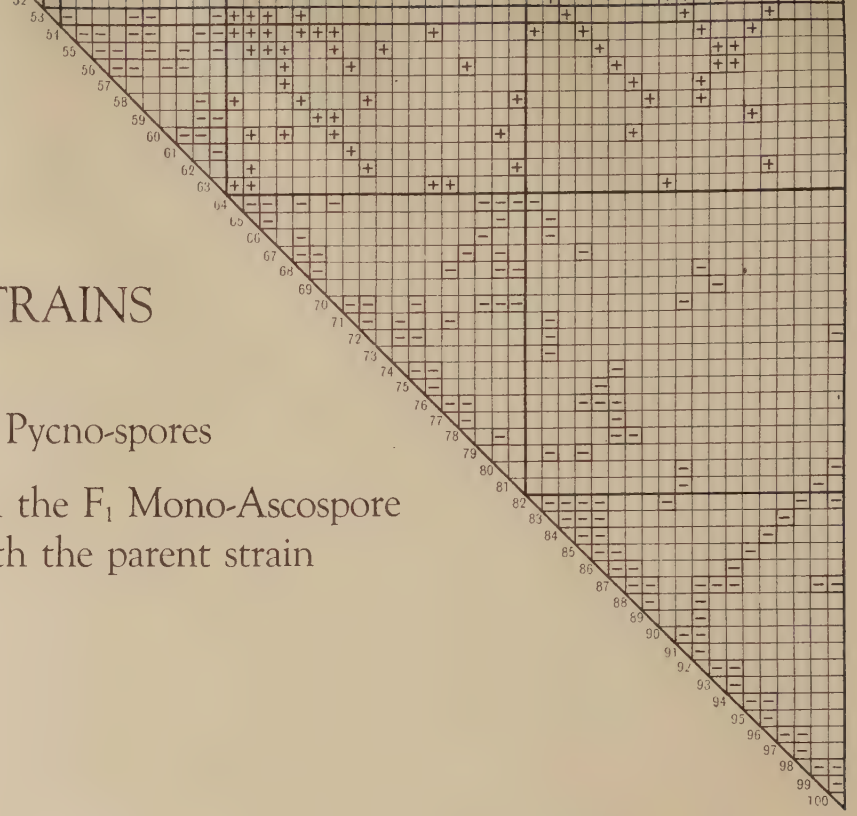
Group I. Strains 1–3. Strains 1 and 2 are *mono-ascospore* strains, and 3 is a *whole ascus* culture, from different perithecia on the same host. It will be seen that strain 1 shows aversion to 2 and 3, but 2 and 3 meet. All three cultures, although different, behave exactly the same towards all strains from other sources, except in Groups XII and XIII, when 2 and 3 behave in the same way towards the F_1 of 2.

Group II. Strains 5–17. Mono-ascospore cultures obtained from several perithecia which developed in pure culture from a mass culture of ascospores isolated from Coe's Golden Drop plum. The whole group is of the same type; the strains show aversion to all the other strains except the F_1 of strain 19. Strain 19 is included in Group III which proved to be the same as Group II, although isolated from two very different varieties of plum. Thus, where strains of the same type occur on different varieties of host they behave in the same way towards other strains from different sources, and the type of strain is irrespective of the variety of the host. A similar case was found in the mono-pycnospore cultures on such widely different hosts as apricot and Coe's Golden Drop plum.

Group III. Strains 18–21. Mono-ascospore strains isolated from mixed perithecia on bark of Prince of Wales plum. Strain 19 of this

CHART I


Group
Different Vars. of Host
Same Host
F1 of 19
F1 of 2
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
Peach MP
Plum, Coe's Golden Drop MP
Pr. of Wales MP
Seedling Plum L1 SP
"
S. Plum SP
S. Plum SP
" "
F1 19
F1 of 19 SP
F1 of 2 SP
F1 of 2 SP
⊞ =Aversion
⊟ =No aversion
MP=Mixed perithecia, same host



CLASS 2

MONO-ASCOSPORE STRAINS

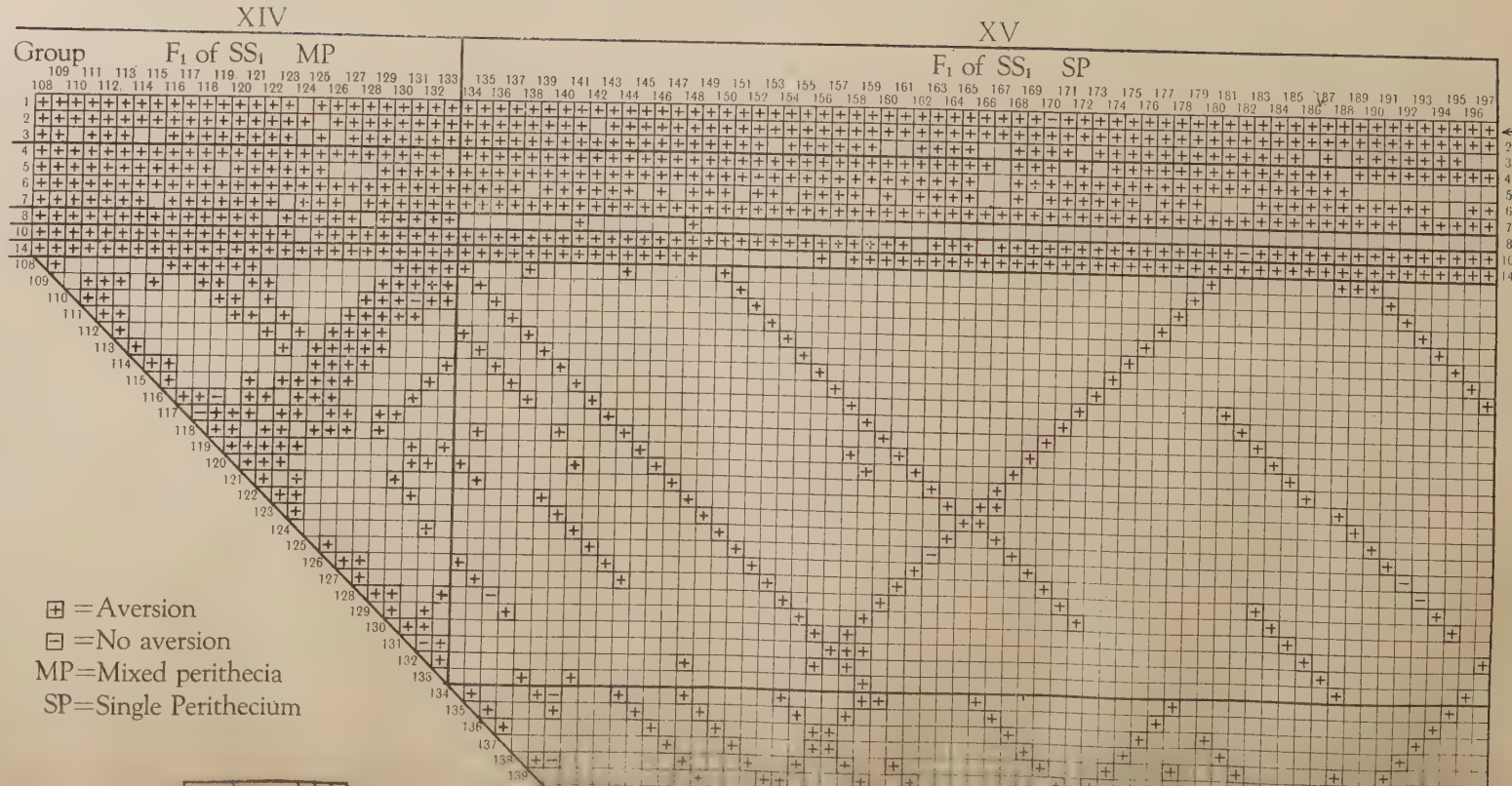
with

Incomplete Life-History i.e. no "b" Pycno-spores

No Aversion in the same perithecium or when the F_1 Mono-Ascospore Mycelia are tested with each other or with the parent strain

See strains 2 and 19

CHART II


XIV
Group
F1 of SS1 MP
XV
F1 of SS1 SP
←SS1
⊞ =Aversion
⊟ =No aversion
MP=Mixed perithecia
SP=Single Perithecium

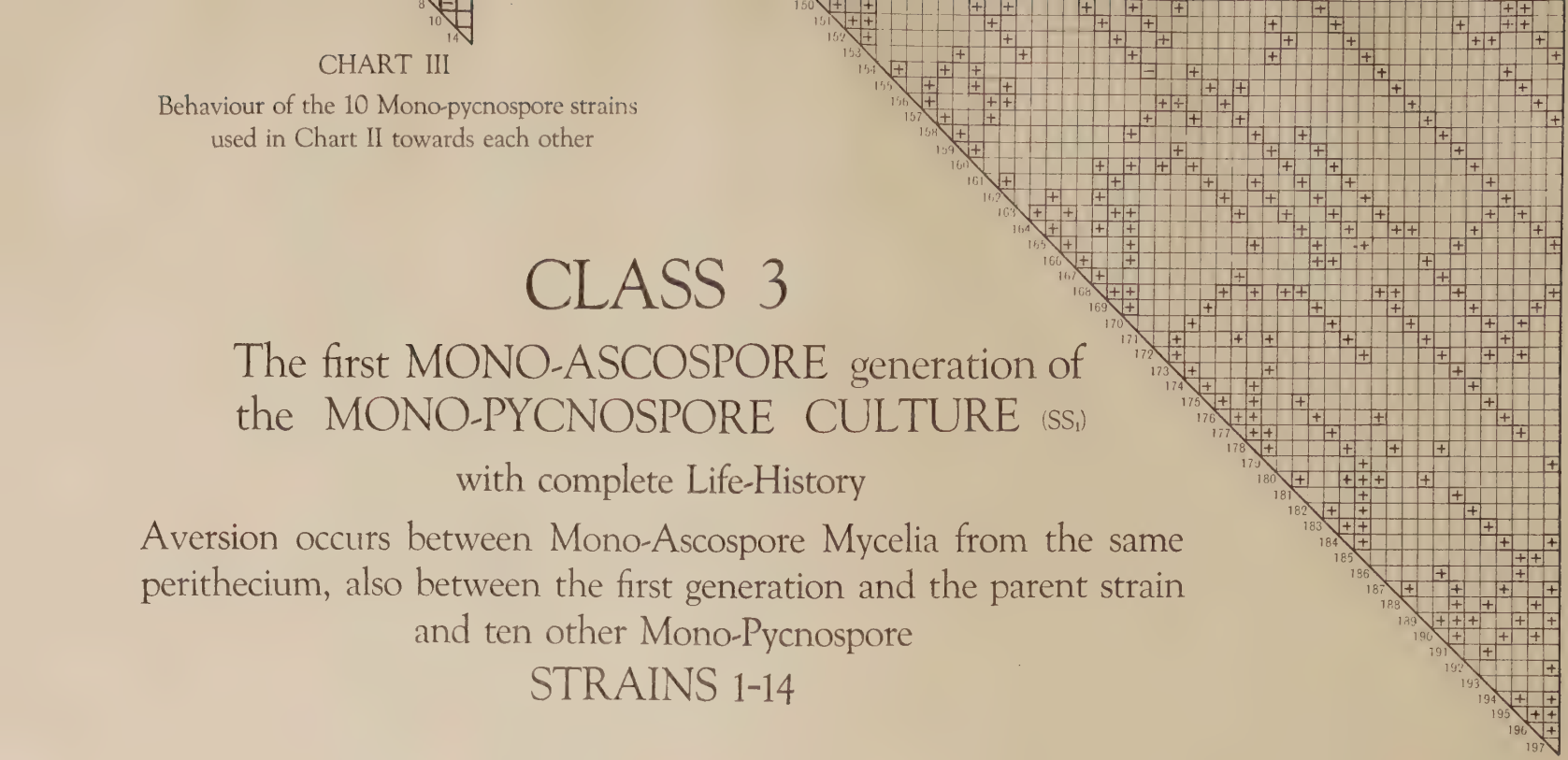


CHART III

Behaviour of the 10 Mono-pycnospore strains
used in Chart II towards each other

CLASS 3

The first MONO-ASCOSPORE generation of the MONO-PYCNOSPORE CULTURE (SS_1)

with complete Life-History

Aversion occurs between Mono-Ascospore Mycelia from the same perithecium, also between the first generation and the parent strain and ten other Mono-Pycnospore

STRAINS 1-14

group shows aversion to all other strains except its own F_1 and Group II. All the other strains in this Group III behave in the same way.

Group IV. Mono-ascospore cultures from the same perithecium on a seedling plum New Orleans × Victoria.

Group V. From the same host as Group IV, but on a different branch. These two groups, IV and V, show aversion to one another, although they occur on the same host.

Groups VI, VII and *VIII.* Isolated from three different perithecia on the stem of a young standard tree of seedling plum from Victoria selfed. One perithecium (Group VI) contains one type of strain and the other two groups are of another type, and yet all three behave the same towards all the groups from other sources.

Groups IX, X, XI are all F_1 of strain 19 from three separate perithecia. They all behave alike, and show aversion to strain 2 and the F_1 of strain 2, and no aversion *inter se* or to their own parent.

Groups XII, XIII. F_1 of strain 2 from two separate perithecia. They also show aversion to 19 and the F_1 of 19, but no aversion *inter se* or to their own parent.

CHART II. (Class 3, Table I, p. 360.)

Group XIV. Mono-ascospore strains 108–110, 116–133, and *mono-ascus* strains (111)–(115) all isolated from mixed perithecia which developed in *mono-pycnospore* culture SS_1 (i.e. the F_1 of SS_1).

All the above cultures (108–133) show aversion towards the parent strain SS_1. On the F_1 strains a few meet, but the majority show aversion *inter se,* and most of them show aversion towards the ten *mono-pycnospore* cultures with which they were tested.

Group XV. Mono-ascospore strains 134–197 are all from the same perithecium in *mono-pycnospore* culture SS_1. It will be seen that a few meet, but the majority show aversion *inter se.* The combinations were made somewhat at random, but in sufficient numbers to give some indication of the behaviour of the F_1 strains towards one another.

CHART III. (Class 1, Table I, p. 360).

Shows the behaviour of the ten stock *mono-pycnospore* cultures used in Chart II, including SS_1. It will be noticed that there is one case of aversion in the same pycnidium, namely SS_4, which shows aversion towards SS_7.

Discussion on heterothallism and sex in the Fungi.

During recent years a considerable amount of work has been published dealing with heterothallism and sex in fungi. The work of Blakeslee (3–7) on the lower fungi is so well known that it need not be very fully discussed here, but it is interesting to note that in the lower fungi, as also in the Ustilaginales (15)—a lower group of the Basidiomycetes belonging to the higher fungi—sex is of a simple character, whereas in the Hymenomycetes—a group of the higher Basidiomycetes—it is of a complex nature.

Blakeslee could divide his Mucors into homo- and heterothallic forms; if heterothallic, the mycelia were either +, or −, or neutral, the latter being a combination of both + and −. If + and − strains are grown together the mycelia will fuse, two cells conjugate to form each zygospore, and a line of zygospores develops where the colonies meet. No zygospores will form if both colonies are of the same sex. The neutral strains will not combine with either + or −.

In the homothallic forms zygospores will develop on the same mycelium.

In the Ustilaginales or smuts two forms of spores are known, the intercalary chlamydospores and the sporidia or basidiospores. The sporidia conjugate in pairs, one nucleus passes over from one spore to the other, but the nuclei do not fuse. Germination then takes place, giving rise to a mycelium in the diplo-phase, with two nuclei in each cell. This condition persists until the development of the mature chlamydospore. The immature chlamydospore also contains two nuclei; these nuclei eventually fuse, so that the mature chlamydospore is mono-nucleate but diploid. The diploid chlamydospore germinates to form a structure called the promycelium (or basidium); the zygote nucleus of the developing promycelium divides twice, the first division being the reduction division, the second the homo-type division. One of the four daughter nuclei passes in each of the four sporidia given off from the promycelium. Kniep (15) working on a number of different forms of smuts has been able to show that in some forms, segregation into two sexes takes place at the reduction division. He does not appear to have isolated the four sporidia from a single promycelium, but the aggregate of a large number of isolations of single sporidia showed that only two sexes occurred and that in some cases, but not in all, the sexes were approximately equal in number. No neutral sporidia were found.

Sporidia are capable of budding and forming colonies when grown in pure culture on an artificial medium, somewhat after the manner of a

yeast, and all the secondary sporidia from a single sporidium proved to be of the same sex and would not conjugate, showing that segregation must have occurred before the development of the sporidia.

In the Hymenomycetes however sex is complicated by the fact that more than two types of sex can occur in one and the same fruiting body. In 1918 Mlle Bensaude (1), working on *Coprinus fimentarius*, was the first to show that heterothallism occurs in the Hymenomycetes, and the following year Kniep found heterothallism in *Schizophyllum commune* and a number of other Hymenomycetes.

In the heterothallic forms the mycelia fuse at an early stage; there is interchange of nuclei, giving rise to the diplo-phase with two nuclei (the dicaryons) in each cell. As in the Ustilaginales these nuclei do not fuse. Later clamp connections form on the young mycelium, short hooked processes which bend over so that their tips fuse with the hyphae from which they originate. Kniep (14) investigated the behaviour of these dicaryons in the region of the clamp connection. He observed that the nucleus nearest the apex of the hypha wanders into the clamp connection, the two nuclei divide conjugately and one half of each nucleus passes into the apical portion of the hyphae, to form the dicaryons of the newly formed cell. Thus the dicaryons which eventually pass into the young basidium are only distantly related to one another. In the basidium the dicaryons fuse, and the zygote nucleus then undergoes two divisions, as in the promycelium of the Ustilaginales.

Mlle Bensaude's criteria of sex in the Hymenomycetes are, the formation of clamp connections, the presence of dicaryons, conjugate division, and the formation of fruiting bodies. Kniep working independently observed clamp connections, dicaryons, and conjugate division in a number of other Hymenomycetes (12–14, 16, 17) but he also found that mono-sporous mycelia of heterothallic forms (12) were sometimes capable of producing mature fruiting bodies in the haploid condition without the intervention of any other strain. In such cases no clamp connections were produced, the cells were mono-nucleate and the spores were all of the same sex.

In homothallic forms clamp connections and dicaryons are always present, the outward and visible signs that the mycelium is in the diplo-phase.

Irene Mounce (19, 20) working on heterothallism in a number of species of the genus *Coprinus* came to the same conclusions as Kniep, namely, that heterothallism is common in the Hymenomycetes and that sex in this group is of a complex character, there being more than two

types. Her tables of combinations of different mono-sporous mycelia agree very closely with those published by Kniep. These conclusions were arrived at from the results of a number of mono-sporous mycelia from the same and from different fruiting bodies. It was not, however, until Kniep(17) was fortunate enough to find a Hymenomycete (*Aleurodiscus polygonius*) from which he could isolate the four basidiospores from a single basidium, that the nature of this complex form of sex could be determined. In *Aleurodiscus*, under cool conditions, each individual basidium sheds its four spores at the same time and the different basidia sporulate in succession, so that without much difficulty the four spores of a basidium can be isolated with certainty. In this fungus Kniep found that segregation into sexes took place at the first nuclear division of the diploid nucleus of the basidium. The diploid nucleus of the basidium divides twice, the first division being the reduction division, the second the homo-type division, giving rise to four nuclei. The basidium then forms four basidiospores, and one haploid nucleus passes into each spore. These four basidiospores germinate and form mycelia, two of which are of one sex and two of the other. He tested a large number of "Vierer-gruppen," or groups of four, from individual basidia, and invariably found that two and only two types of sex occurred in spores from the *same* basidium, and that the sexes

TABELLE I[1].

	1	2	3	4
1	−	−	+	+
2	−	−	+	+
3	+	+	−	−
4	+	+	−	−

One " Vierer-gruppe."

segregated out in equal numbers (17) Tabelle I, p. 11. He then combined the four mycelia from *one* basidium with

(i) Mycelia from *other* basidia on the *same* fruiting body.

[1] In this, and the following Tables, the signs do not denote sex. The + sign indicates that fusion has taken place between haploid mycelia of different sexes, and the − sign indicates no fusion.

(ii) Mycelia from *different* fruiting bodies from the same spot and from different localities,

to see if the "Vierer-gruppen" were identical.

The results could be grouped under three headings.

A. From the *same* fruiting body.

(1) Fusion in pairs, in which case the four-groups are identical. Tabelle I.

(2) No fusion at all.

B. From *different* fruiting bodies.

(3) Fusion of all mycelia however combined, or the reverse.

He then combined the "four-groups" from *different* basidia with 10 known strains from the *same* fruiting body, with the results shown in his table below.

TABELLE II (17, p. 11).

	1	2	3	4	5	6	7	8	9	10
3*a*	−	+	−	−	−	−	−	−	−	−
3*b*	−	+	−	−	−	−	−	−	−	−
3*c*	−	−	−	−	+	−	−	+	−	+
3*d*	−	−	−	−	+	−	−	+	−	+
5*a*	−	−	−	−	−	−	+	−	+	−
5*b*	−	−	−	−	−	−	+	−	+	−
5*c*	+	−	+	+	−	+	−	−	−	−
5*d*	+	−	+	+	−	+	−	−	−	−
10*a*	−	−	−	−	−	−	+	−	+	−
10*b*	−	−	−	−	−	−	+	−	+	−
10*c*	+	−	+	+	−	+	−	−	−	−
10*d*	+	−	+	+	−	+	−	−	−	−

Three "Vierer-gruppen" (i.e. 3, 5 and 10) combined with 10 known strains from the *same* fruiting body.

It can be seen at a glance that there are four distinct types of sex, and Kniep so far has always found four, and never more than four, types in the same fruiting body in *Aleurodiscus polygonius.*

Assuming that all the zygote nuclei in the basidia of the same fruiting body are identical, Kniep offers the following explanation.

Since four different haplonts occur, then the basidia in the diploid phase cannot be homozygous, and sex must depend upon two pairs of factors, *Aa* and *Bb*. The zygote in the basidium will therefore be *AaBb*, and the four possible gametes or haplonts *AB*, *ab*, *Ab*, *aB*. Out of 16 possible combinations, there are only nine genotypically different diplonts, four of which are homozygous in both factors, four homozygous in one factor, giving two different haplonts, and one heterozygous in both factors, giving the four different haplonts from which we started. As four different forms of sex mycelia are obtained from one fruiting body, only those combinations can occur in which the resulting zygote is heterzygous in both factors (selective fertilisation). The genes in one basidium segregate out to form spores of the constitution *AB* and *ab* (Tabelle III) and in another basidium *Ab*, *aB* (Tabelle IV). Kniep found that these two types of basidia occurred in approximately equal numbers. Each of these types should be capable of fusion of mycelia *inter se*, but not with the other type, for the haplonts of these basidia cannot form diplonts heterozygous in both factors, and therefore, as Kniep's results showed, no fusion can occur between them.

TABELLE III.

		1	2	3	4
		AB	*AB*	*ab*	*ab*
1	*AB*	−	−	+	+
2	*AB*	−	−	+	+
3	*ab*	+	+	−	−
4	*ab*	+	+	−	−

Basidium I.

TABELLE IV.

		1	2	3	4
		Ab	*Ab*	*aB*	*aB*
1	*Ab*	−	−	+	+
2	*Ab*	−	−	+	+
3	*aB*	+	+	−	−
4	*aB*	+	+	−	−

Basidium II.

Different basidia from the *same* fruiting body.

To explain the fusion of all mycelia from *different* fruiting bodies (B 3, p. 367), Kniep suggests multiple allelomorphism, but he points out that he has no definite proof of this in *Aleurodiscus*, as, so far, he has not been able to obtain the hybrid F_1 fruiting body (F_1 sporophyte) from combinations of haplonts from different fruiting bodies. His experiments with *Schyzophyllum commune* populations, however, have

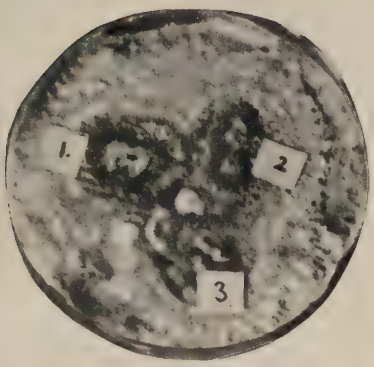


Fig. 4. $2+F_1$ of $2+F_1$ of 2.

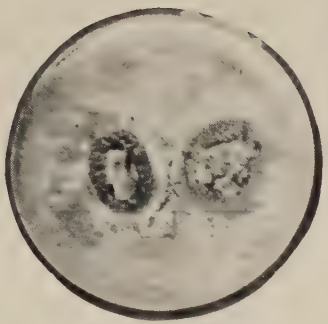


Fig. 1. $19+19$.

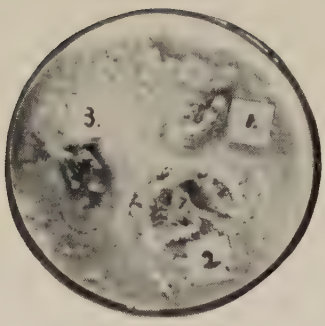


Fig. 7. $19+F_1$ of $19+F_1$ of 19.

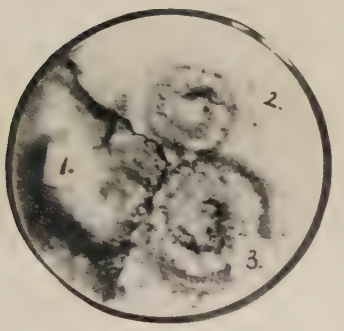


Fig. 5. F_1 of $2+F_1$ of $19+F_1$ of 19.

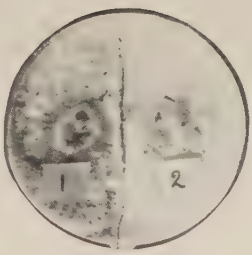


Fig. 2. $2+9$.

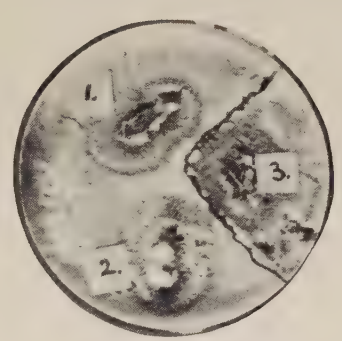


Fig. 8. $19+F_1$ of $19+F_1$ of 2.

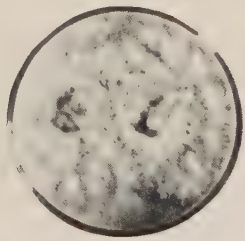


Fig. 3. $2+2$.

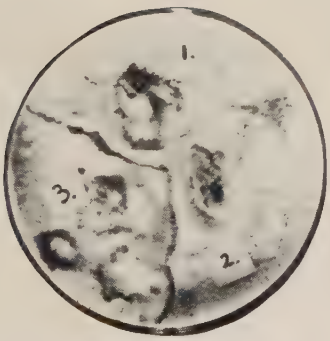


Fig. 6. F_1 of $2+F_1$ of $2+F_1$ of 19.

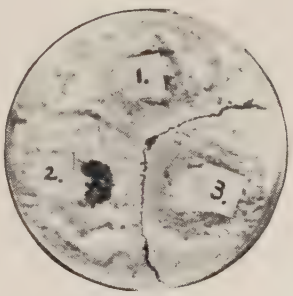


Fig. 9. $19+F_1$ of $19+F_1$ of 2.

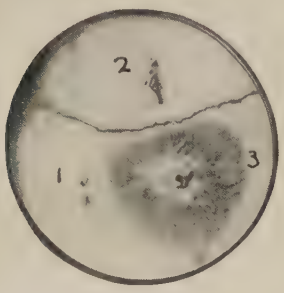


Fig. 1.

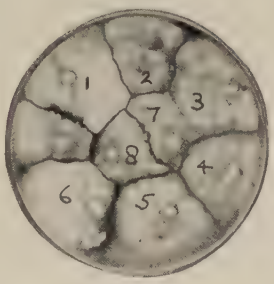


Fig. 5.

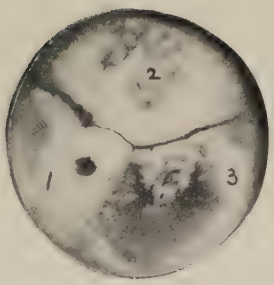


Fig. 2.

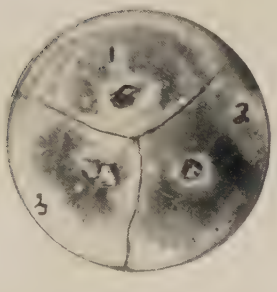


Fig. 3.

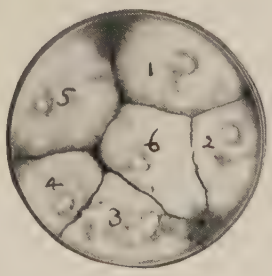


Fig. 6.

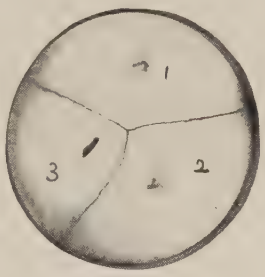


Fig. 4.

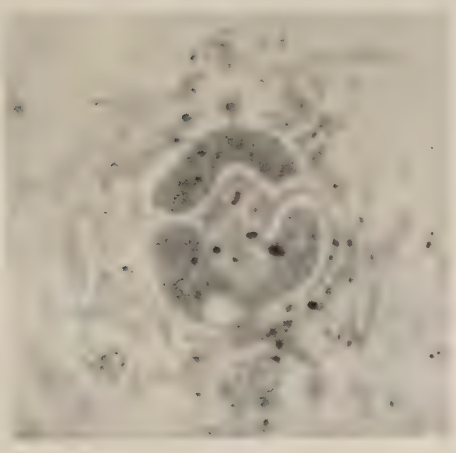


Fig. 7.

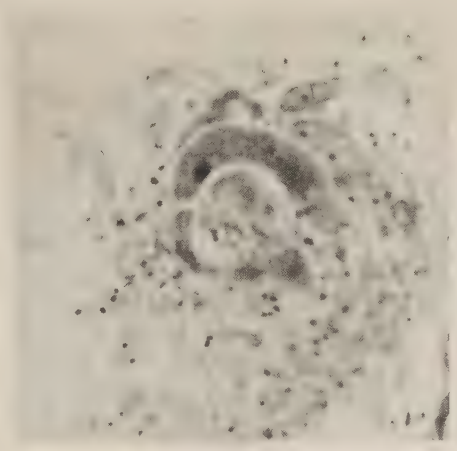


Fig. 8.

Fig. 9.

led him to adopt this explanation. In this fungus he raised a number of hybrid F_1 sporophytes and from these he isolated mono-spore mycelia (F_1-gametophytes) and found (in some cases but not in all) four types of sex in the haploid F_1-generation.

In conclusion I wish to express my thanks to Prof. H. Kniep for his courtesy in allowing me to reproduce some of his Tables.

EXPLANATION OF PLATES.

PLATE XIX.

Mono-ascospore cultures included in Class 2. (Table I, p. 360.)

Fig. 1. Culture SA_{19} combined with itself. (Parent strain.) *No aversion.*

Fig. 2. Culture SA_{19} combined with SA_2. *Aversion.*

Fig. 3. Culture SA_2 combined with itself. (Parent strain.) *No aversion.*

Fig. 4. Culture SA_2 combined with two colonies of its F_1. *No aversion.*

Fig. 5. F_1 of SA_2 (col. 1) combined with two colonies of the F_1 of SA_{19} (colonies 2 and 3). *Aversion.*

Fig. 6. Two colonies of the F_1 of SA_2 (cols. 1 and 2) combined with one colony of F_1 of SA_{19}. *Aversion.*

Fig. 7. Culture SA_{19} (col. 1) combined with two colonies of its own F_1 (cols. 2 and 3). *No aversion.*

Fig. 8. Culture SA_{19} (col. 1) combined with one colony of its own F_1 (col. 2) and one colony of the F_1 of SA_2 (col. 3). *Aversion.*

Fig. 9. Culture SA_{19} (col. 1) combined with one colony of its own F_1 (col. 2) and one colony of the F_1 of SA_2 (col. 3). *Aversion.*

PLATE XX.

Mono-ascospore cultures included in Class 3. (Table I, p. 360).

Fig. 1. Two F_1 mono-ascospore mycelia of SS_1 (cols. 2 and 3) combined with stock mono-pycnospore SS_{10} (see Charts II and III). SS_{10} *meets* col. 3 but shows *aversion* to col. 2.

Fig. 2. Three F_1 mono-ascospore mycelia of SS_1. Cols. 2 and 3 are from the same perithecium, col. 1 from another perithecium.

Fig. 3. Three F_1 mono-ascospore mycelia of SS_1 from the same perithecium. *General aversion.*

Fig. 4. Two F_1 mono-ascospore mycelia of SS_1 (cols. 2 and 3) combined with the parent mono-pycnospore culture (col. 1). *General aversion.*

Fig. 5. Eight mono-ascospore mycelia of SS_1 } from the same perithecium. *General*
Fig. 6. Six mono-ascospore mycelia of SS_1 } *aversion.*

Figs. 7 and 8. Two consecutive sections through a young perithecium in SS_1 on oat-agar, showing archicarp.

Fig. 9. Section through line of demarcation between two averting strains on oat-agar.

(*a*) aerial mycelium.
(*b*) submerged mycelium.
(*c*) crack in the agar.

The micro-photographs on Plate II were taken by Mr Osterstock, and the rest of the photographs by A. F. Emarton, laboratory assistant in this Institution.

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